CHEMICAL AND BIOLOGICAL DEFENSE PROGRAM
SBIR 08.1 Proposal Submission

General Information

In response to Congressional interest in the readiness and effectiveness of U.S. Nuclear, Biological and Chemical (NBC) warfare defenses, Title XVII of the National Defense Authorization Act for Fiscal Year 1994 (Public Law 103-160) required the Department of Defense (DoD) to consolidate management and oversight of the Chemical and Biological Defense (CBD) Program into a single office – Office of the Special Assistant, Chemical and Biological Defense and Chemical Demilitarization Programs. The Joint Science and Technology Office for Chemical and Biological Defense (JSTO-CBD), Defense Threat Reduction Agency (DTRA) provides the management for the Science and Technology component of the Chemical and Biological Defense Program. Technologies developed under the SBIR program have the potential to transition to the Joint Program Executive Office for Chemical and Biological Defense (JPEO-CBD) if the appropriate level of technology maturity has been demonstrated. The JSTO-CBD Science & Technology programs and initiatives are improving defensive capabilities against Chemical and Biological Weapons. The Small Business Innovation Research (SBIR) portion of the CBD Program is managed by the JSTO-CBD through the Army SBIR Program Management Office (PM, SBIR), Ft. Belvoir, VA.

The mission of the Chemical and Biological Defense Program is to ensure that the U.S. military has the capability to operate effectively and decisively in the face of biological or chemical warfare threats at home or abroad. Numerous rapidly-changing factors continually influence the program and its management, including planning for war-fighting support to asymmetrical threats, the evolving geopolitical environment, U.S. participation in the Chemical Weapons Convention, the threat of global proliferation of chemical and biological weapons, and DoD resources available. Improved defensive capabilities are essential in order to minimize the impact of such weapons. U.S. forces require aggressive, realistic training and the finest equipment available that allows them to avoid contamination, if possible, and to protect, decontaminate and sustain operations. Further information about the DoD CBD Program (and related programs) is available at the DoD Counter proliferation and Chemical Biological Defense Homepage at http://www.acq.osd.mil/cp.

The overall objective of the CBD SBIR Program is to improve the transition or transfer of innovative CBD technologies between DoD and the private sector for mutual benefit. The CBD SBIR Program targets those technology efforts that maximize a strong defensive posture in a biological or chemical environment using passive and active means as deterrents. These technologies include chemical and biological detection; individual and collective protection; decontamination; information systems technology; threat agent science; and medical pre-treatments, diagnostics, and therapeutics.

Submitting Your Phase I CBD SBIR Proposal

Your entire proposal (consisting of Proposal Cover Sheets, the full Technical Proposal, Cost Proposal, and Company Commercialization Report) must be submitted electronically through the DoD SBIR/STTR Proposal Submission system located at www.dodsbir.net/submission. A hardcopy is NOT required for CBD. Hand or electronic signature on the proposal is also NOT required.

You must prepare a Company Commercialization Report through the Submission site and it will be included with your electronic submission; however, it does not count against the proposal page limit. Update your commercialization information if you have not done so in the past year. Please note that improper handling of the Commercialization Report may result in the proposal being substantially delayed and that information provided may have a direct impact on the review of the proposal. Refer to section 3.5d at the program solicitation for detailed instructions on the Company Commercialization Report.

Be reminded that section 3.5.a of this solicitation states: "If your proposal is selected for award, the technical abstract and discussion of anticipated benefits will be publicly released on the Internet; therefore, do not include proprietary or classified information in these sections". Note also that the DoD web site contains timely information on firm, award, and abstract data for all DoD SBIR Phase I and II awards archived for several years. This information can be viewed on the DoD SBIR/STTR website at http://www.acq.osd.mil/sadbu/sbir/.

The CBD SBIR Program has enhanced its Phase I-Phase II transition process by implementing the use of a Phase I Option that may be exercised to fund interim Phase II activities while a Phase II contract is being negotiated. The maximum dollar amount for a Phase I feasibility study is $70,000. The Phase I Option, which **must** be proposed as part of the Phase I proposal, covers activities over a period of up to three months and at a cost not to exceed $30,000. All proposed Phase I Options must be fully costed and should describe appropriate initial Phase II activities, which would lead, in the event of a Phase II award, to the successful demonstration of a product or technology. **The CBD SBIR program will not accept Phase I proposals which exceed $70,000 for the Phase I effort and $30,000 for the Phase I Option effort**. Only those Phase I efforts selected for Phase II awards through the CBD SBIR Program's competitive process will be eligible to exercise the Phase I Option. To maintain the total cost for SBIR Phase I and Phase II activities at a limit of $850,000, the total funding amount available for Phase II activities under a resulting Phase II contract will be $750,000.

Companies submitting a Phase I proposal under this Solicitation must complete the Cost Proposal using the on-line form within a total cost of $70,000 over a period of up to 6 months (plus up to $30,000 for the Phase I Option over a period of up to three (3) months). Phase I and Phase I Option costs must be shown separately.

Selection of Phase I proposals will be based upon scientific and technical merit, according to the evaluation procedures and criteria discussed in section 4.2. The CBD SBIR Program reserves the right to limit awards under any topic, and only those proposals of superior scientific and technical quality in the judgment of the technical evaluation team will be funded.

Proposals not conforming to the terms of this solicitation, and unsolicited proposals, will not be considered. Awards are subject to the availability of funding and successful completion of contract negotiations.

CBD Program Phase II Proposal Guidelines

Phase II is the demonstration of the technology that was found feasible in Phase I. Only those Phase I awardees which achieved success in Phase I, as determined by the project technical monitor measuring the results achieved against the criteria contained in section 4.3, will be invited to submit a Phase II proposal. During or at the end of the Phase I effort, awardees will be invited to submit proposals for evaluation for a Phase II award. The invitation will be issued in writing by the organization responsible for the Phase I effort. Invited proposers are required to develop and submit a commercialization plan describing feasible approaches for marketing the developed technology. Proposers are required to submit a budget for the entire 24 month Phase II period. During contract negotiation, the contracting officer may require a cost proposal for a base year and an option year, thus, proposers are advised to be mindful of this possibility. These costs must be submitted using the Cost Proposal format (accessible electronically on the DoD submission site), and may be presented side-by-side on a single Cost Proposal Sheet. The total proposed amount should be indicated on the Proposal Cover Sheet as the Proposed Cost. At the Contracting Officer's discretion, Phase II projects may be evaluated after the base year prior to extending funding for the option year.

The CBD SBIR Program is committed to minimizing the funding gap between Phase I and Phase II activities. All CBD SBIR Phase II proposals will receive expedited reviews and be eligible for interim funding (refer to top for information on the Phase I Option). Accordingly, all Phase II proposals will be evaluated within a single two-tiered evaluation process and schedule. Phase II proposals will thus typically be submitted within 5 months from the scheduled DoD Phase I award date (the scheduled DoD award date for Phase I, subject to the Congressional Budget process, is 4 months from close of the DoD Solicitation). The CBD Program typically funds a cost plus fixed fee Phase II award, but may award a firm fixed price contract at the discretion of the Contracting Officer.

Key Dates

08.1 Solicitation Open/Close	10 December 2007 – 9 January 2008
Phase I Evaluations	January - March 2008
Phase I Selections	March 2008
Phase I Awards	May 2008*
Phase II Invitations	September 2008
Phase II Proposals due	October 2008

*Subject to the Congressional Budget process.

CBD SBIR PROPOSAL CHECKLIST

This is a Checklist of Requirements for your proposal. Please review the checklist carefully to ensure that your proposal meets the CBD SBIR requirements. **Failure to meet these requirements will result in your proposal not being evaluated or considered for award**.

_____ 1. The Proposal Cover Sheets along with the Technical Proposal, Cost Proposal and Company Commercialization Report were submitted via the Internet using the DoD's SBIR/STTR Proposal Submission website at http://www.dodsbir.net/submission.

_____ 2. The proposal cost adheres to the CBD Program criteria specified.

_____ 3. The proposal is limited to only **ONE** solicitation topic. All required documentation within the proposal references the same topic number.

_____ 4. The Project Abstract and other content provided on the Proposal Cover Sheet contains no proprietary or classified information and is limited to the space provided.

_____ 5. The Technical Content of the proposal, including the Option (if applicable), includes the items identified in Section 3.4 of the solicitation.

_____ 6. The Proposal Cover Sheets and technical proposal is 25 pages or less in length. The Cost Proposal and Company Commercialization Report do not count against the 25 page limit. Pages in excess of this length will not be considered for review or award.

_____ 7. The Company Commercialization Report is submitted online in accordance with Section 3.5.d. This report is required even if the company has not received any SBIR funding

_____ 8. The proposal contains no type smaller than 10-point font size (except as legend on reduced drawings, but not tables).

CBD SBIR 08.1 Topic Index

CBD SBIR 08.1 Topic Descriptions

CBD08-101 TITLE: Rapid, High Resolution Protein Separation System

TECHNOLOGY AREAS: Chemical/Bio Defense, Biomedical

OBJECTIVE: The objective of this research is to produce a high resolution system whereby proteins present in a complex mixture can be rapidly and reproducibly separated. The system should have the potential to be applied to global proteomics analyses. “Fingerprints” from global proteomics can be used to identify biological agents and identify changes in cellular proteins in soldiers after exposure to stressors, thereby monitoring soldiers’ physiological health.

DESCRIPTION: The ability to obtain rapid and sharp separation of proteins from complex mixtures is an essential tool for biological research. Two dimensional gel electrophoresis (2DGE) is the most common approach and separates proteins in one dimension based on charge, and in the other based on size. The technology works as follows: a protein extract is mixed with ampholytes and is subjected to an electric field. The proteins migrate to their isoelectric points within the electric field. They are subsequently size fractionated by electrophoresis through a polyacrylamide gel. Although 2DGE provides enormous separation resolution with the ability to separate thousands of proteins, this approach is limited by poor reproducibility and labor-intensity.

The goal of this topic is to build on recent advances in macromolecule separations technologies. Microfluidics can be used for separations of biologicals including amino acids (1), DNA (2) and proteins (3) by exploiting their individual properties. Proteins and peptides have been preconcentrated (4), sorted by their isoelectric points (5,6) or size (7,8) using microfluidics. In fact, proteome analyses of relatively simple systems have already been undertaken (9,10). For this topic, research will focus on developing a device that can provide rapid and sharp separation of proteins and has the potential to eventually be applicable to global protein analysis. This may include significant improvements of existing technologies, newly developed technology, or combinations of the two.

PHASE I: Initial research will be directed at providing proof of concept that a microfluidics-based system can separate all the individual proteins from a fairly simple mixture in a rapid (<10 minutes) and reproducible manner. The theory for separation and method of protein detection should be well established. A brassboard will be produced and is expected to be capable of resolving all proteins from a mixture of 20-30 proteins that vary in size and charge by as little as 10% of their molecular weights and 0.1 pI units (pI = isoelectric point).

PHASE II: The deliverable at the end of Phase II is expected to be a device that can rapidly (<10 minutes) separate biologically relevant proteins in the molecular weight range of 16-200 Kd (kilodalton) and pI range of 3-12 from a complex mixture, such as a human cell homogenate, in a reproducible manner. Research during this phase will expand on the results from Phase I. Experimental conditions will be well defined and result in consistent, reproducible protein separations that approach the resolution of 2DGE. The resulting device should be fieldable (i.e., portable enough that it could be used in a forward medical hospital setting).

PHASE III DUAL USE APPLICATIONS: Development of a device to rapidly and reproducibly conduct proteomics analyses will allow the development of proteomic “fingerprints” of biological pathogens and potential biowarfare agents which in turn will facilitate agent identification in the event of a bioterrorism attack or a disease outbreak. In addition to Dodd and Homeland Security applications of this technology for proteomics-based detection and classification of biological warfare agents, monitoring physiological health status, and proteomics-based diagnostics there are potential civilian applications to include point-of-care diagnostics, pharmaceutical drug discovery, biological research (basic and applied), forensics and all other uses that are currently fulfilled by 2DGE instrumentation.

REFERENCES:

1. Pumera M. Microfluidics in amino acid analysis. 2007 Electrophoresis. In press.

2. Regtmeier J, Duong TT, Eichhorn R, Anselmetti D, Ros A. 2007. Dielectrophoretic manipulation of DNA: separation and polarizability. Anal Chem. 79(10):3925-32.

3. Ros A, Hellmich W, Regtmeier J, Duong TT, Anselmetti D. 2007. Bioanalysis in structured microfluidic systems. Electrophoresis. 27(13):2651-8.

4. Hatch AV, Herr AE, Throckmorton DJ, Brennan JS, Singh AK. 2007. Integrated preconcentration SDS-PAGE of proteins in microchips using photopatterned cross-linked polyacrylamide gels. Anal Chem. 78(14):4976-84.

5. Song YA, Hsu S, Stevens AL, Han J. 2006. Continuous-flow pI-based sorting of proteins and peptides in a microfluidic chip using diffusion potential. Anal Chem. 78(11):3528-36.

6. Das C, Fan ZH. 2006. Effects of separation length and voltage on isoelectric focusing in a plastic microfluidic device. Electrophoresis. 27(18):3619-26.

7. Agirregabiria M, Blanco FJ, Berganzo J, Fullaondo A, Zubiaga AM, Mayora K, Ruano-López JM. SDS-CGE of proteins in microchannels made of SU-8 films. Electrophoresis. 27(18):3627-34.

8. Dodge A, Brunet E, Chen S, Goulpeau J, Labas V, Vinh J, Tabeling P. 2006. PDMS-based microfluidics for proteomic analysis. Analyst. 131(10):1122-8.

9. Mann AM, Tighe BJ. 2007. Tear analysis and lens-tear interactions Part I. Protein fingerprinting with microfluidic technology. Cont Lens Anterior Eye. In press.

10. Thongboonkerd V, Songtawee N, Sritippayawan S. 2007. Urinary proteome profiling using microfluidic technology on a chip. J Proteome Res. 6(5):2011-8.

KEYWORDS: protein, separation, proteomics, microfluidics, two dimensional gel electrophoresis

CBD08-102 TITLE: <u>Reduced Fluorescence in a Handheld Chemical Identification System using Raman Spectroscopy at Wavelengths greater than 950 nm</u>

TECHNOLOGY AREAS: Chemical/Bio Defense, Biomedical

ACQUISITION PROGRAM: CUGR Advanced Concepts Technology Demonstration (ACTD)

OBJECTIVE: To develop and field a light weight, hand held non-contacting, chemical identification system utilizing Raman spectroscopy at wavelengths greater than 950 nm. The intent is to reduce the deleterious effects of molecular fluorescence contributed from both impurities and target analytes. Current systems exist and are fielded using laser diode sources at 785 nm. However, these systems are inadequate as their Raman spectra show significant interference from fluorescence. The fluorescence signal corrupts the information in the Raman spectrum reducing the probability of detection (PD) and in some cases increases the probability of false alarm (PFa).

DESCRIPTION: The specific objectives of the topic follows:

1. Modify and/or adapt a commercial handheld Raman system for use with a laser source greater than 950 nm.
2. Specific modeling goals include:
   a. Comparison of the Raman and fluorescence signals of specific target analytes acquired at 785 nm and at wavelengths > 950 nm.

b. Prediction of optimal experimental conditions and instrumental parameters to collect Raman spectra at wavelengths > 950 nm.
c. Estimation of the effect of laser wavelength on the PD and PFa in spectral matching algorithms.
3. Demonstration of non-contact chemical identification using Raman spectroscopy at wavelengths greater than 950 nm.
4. Development of a database of the Raman spectra at the ultimate wavelength chosen.
5. Development of statistical classification algorithms to identify and determine the relative concentration of a threat analyte based on its Raman spectrum.

PHASE I: Phase I research will be restricted to demonstrating feasibility of using Raman spectroscopy at wavelengths longer than 950 nm. The effort should include sufficient modeling and experimental data to demonstrate the trade-offs in moving to longer wavelengths and the advantage gained in fluorescence reduction.

PHASE II: Phase II tasks will result in the delivery of a beta system, or at a minimum, a brass-board prototype capable of identifying toxic industrial chemicals, explosive mixtures, and chemical warfare agents. The system should have sufficient algorithm development to complete the data acquisition, preprocessing of the data, statistical analysis of collected spectra and classification analysis in real time and on-board the instrument.

PHASE III Dual Use Applications: Commercial applications of this technology include non-intrusive interrogation of sealed transparent containers for quality assurance, law enforcement and international treaty verification. Law enforcement applications include crime scene investigation; illicit drug identification; inspection of food containers and hazardous waste containers.

REFERENCES:
1. “Comparison of Portable Raman Instruments for Use in the Single CAIS Access and Neutralization (SCANS) Project,” Steven Christesen, Lawrence Procell, David Sorrick, and Brian MacIver, ECBC-TR-102, August 2000. This document is approved for public release distribution unlimited and will be provided upon request.

KEYWORDS: Raman spectroscopy, fluorescence rejection, near infrared, detection, identification

CBD08-103 TITLE: Collective Protection for Military Working Dogs

TECHNOLOGY AREAS: Chemical/Bio Defense, Biomedical

OBJECTIVE: To fill a capability gap for military working dogs, by developing a chemical and biological (CB) protective enclosure to shelter and protect them in the event of an agent attack. Integration of protective materials, thermal and ventilation control concepts will be explored.

DESCRIPTION: Military working dogs have been used since World War II. Dogs are used for such duties as, explosives detection, security and patrol, search and rescue, and guarding1. Dogs are trained and certified, a process that takes approximately 18 months and significant monetary resources. In the event of a CB attack, the dogs must be protected in order to assure continued battlefield operations. Shelter enclosures for military working dogs in the event of a CB attack is a technology gap identified by the Joint Requirements Office (JRO) for Chemical, Biological, Radiological, and Nuclear Defense (CBRND) in the Future Needs Analysis (FNA) Functional Solutions Analysis (FSA)2.

Masks and protective suits are impractical for dogs; a protective enclosure is the best solution for CB protection since dogs can vary anywhere from 60 to 120 lbs. This shelter enclosure could integrate with the current plastic kennels that dogs are transported in or could be a unique shelter to protect the dogs. This shelter should be low weight and cube, to minimize transport requirements. The shelter would allow the handler to put the dog in and protect them in the event of an attack so they would survive for future operations. The shelter should be able to be set up in the time that the handler would be putting on an individual CB suit.

The shelter must be able to accommodate the dog, or dogs, and keep them at a comfortable temperature1. The enclosure must have the capability to operate in temperatures basic cold to basic hot as defined in Army Regulation 70-383. In addition to comfort in extreme temperature the material and the shelter itself must allow for the transfer of oxygen and carbon dioxide. Adequate ventilation rates must be met. The climate control and oxygen transfer devices must minimize weight and logistical burden to the shelter. A non-powered system is optimal. Establishment of detailed system specifications will be determined in Phase I.

Research will encompass all aspects of enclosing a dog in this protective enclosure. Threshold and objective quantitative health requirements including physiological, anatomical and psychological will be defined after consulting with both military and commercial sources. Possible issues to be addressed include the following:

- Target oxygen and carbon dioxide exchange
- Introduction of humidity due to sweating, panting and increased respiration
- Increased core temperature Ideal enclosure dimensions due to weight, size and psychological factors
- Health concerns related to animal waste
- Characterization of optimal lighting levels
- Acoustic limits and behavioral impact

Research being conducted under this topic must comply with Federal and Department of Defense Regulations, and Public Law (in particular Animal Welfare Act 4 and amendments) regarding the treatment of dogs.

Including active and/or passive barrier materials to achieve the best material for the shelter enclosure to protect the dogs until the area is safe. In addition to barrier (passive) materials, which block CBWAs, there are emerging active textiles. These active, smart textiles use CBWAs as a catalyst for self-detoxification and the chemical reaction for decontamination creates a safe environment from CBWAs. A self-detoxifying material by nature should be reusable since the chemical reaction taking place rids the material of any CBWA residue. Both a passive and active barrier material for the shelter should be investigated and the best material will be used in the follow on phases of the program.

The material must pass 72 hours of protection when tested in accordance with Test Operation Procedure (TOP) 8-2-5015, and/or the replacing test procedure for reactive materials, if applicable. The material must be rugged and durable enough to withstand environmental loading, rough handling, and folding but also lightweight in order to have minimal logistical burden of enclosure systems. The material must also be flame restistant5.

PHASE I: Determine system and performance parameters providing the supporting referenced studies and rationale. This might include, but is not limited to, the range of animal sizes, physiological requirements, size/weight requirements for transport, etc. A feasibility study will be conducted to evaluate the potential of CB barrier material, both active and passive. The durability, reactivity, reusability, and logistics, such as upkeep and maintenance, will be investigated for the applicable CB materials. Materials used in construction of the shelter enclosure must be low weight and cube. The shelter enclosure will be required to accommodate a dog or dogs and keep them at a comfortable temperature while transferring oxygen and carbon dioxide without increasing weight or logistical burdens. Potential designs will be created for the overall system concept, and a laboratory study conducted to evaluate its performance and applicability. Demonstrate through experimentation, the feasibility of paring the CB materials into a collectively protected shelter enclosure for this application. At the end of Phase I, a conceptual prototype will be demonstrated.

PHASE II: The Phase I concept, active or passive, will be further optimized for characteristics such as performance durability and fabrication, as a full-scale collective protection shelter system for the military working dogs. The fabrication costs must be kept at a minimum. Means by which the material can be made into a full scale prototype will be assessed. The seaming and/or welding of seams of the material will be explored, tested and optimized. A final full system demonstration will be conducted and evaluated for system performance. In addition, an investigation of potential alternative applications should be conducted in conjunction with a market assessment.

PHASE III DUAL-USE APPLICATIONS: These materials and technology developed under this project could also be integrated into military individual protection equipment and collective protection equipment for the Warfighter. The material would provide real-time chemical reaction with a hazardous environment, enabling the Warfighter to have additional protection from CBWAs. Proposed material could be introduced into the civilian marketplace along with current civilian CB barrier technologies. The shelter could be incorporated for use in other applications for military animals. The shelter would also be applicable to federal, state and local law enforcement. The temperature and ventilation systems used could separately be marketed for government and commercial uses.

REFERENCES:

1. Military Police: Military Working Dog Program, Department of the Army Pamphlet 190-12, Headquarters Department of the Army Washington, DC 30 Sept 1993.

2. Joint Requirements Office Chemical Biological Radiological and Nuclear Defense (JRO-CBRND) Functional Needs Analysis (FNA)/Functional Solution Analysis (FSA). 6 Dec 2005.

3. Research and Development Test and Evaluation of Material for Extreme Climatic Conditions, Army Regulation 70-38, Headquarters Department of the Army Washington, DC 15 September 1979.

4. Animal Welfare Act (7 USC 2131-2156), United States Code, Title 7, Sections 2131 to 2156. 1966, 1970, 1976, 1985, 1990.

5. US Army Test and Evaluations Command Test Opperations Procedure (TOP) 8-2-501, “Permeation and Penetration Testing of Air-Permeable, Semi-Permeable, and Impermeable Materials with Chemical Agents and Simulates (Swatch Testing)”

6. “Limited Production Purchase Description for Cloth, Chemical and Biological Protective” LP/P DES 1-94b, 5 March 2000.

7. Field Manual 3-11, Multi Service Tactics, Techniques, and Procedures for Nuclear, Biological, and Chemical Defense Operations, U. S. Army Training and Doctrine Command, Fort Monroe VA, 10 March 2003.

8. Field Manual 3-11.4, Multi Service Tactics, Techniques, and Procedures for Nuclear, Biological, and Chemical Protection, U. S. Army Training and Doctrine Command, Fort Monroe VA, 1 June 2003.

KEYWORDS: Self-detoxify, Barrier Material, Chemical and Biological Protection, Reactive Materials, Decontamination, Military Working Dogs

CBD08-104 TITLE: Dynamic Multicomponent Optical Analyzer for Chemical Weapon (CW) Exposure Studies

TECHNOLOGY AREAS: Chemical/Bio Defense, Biomedical

OBJECTIVE: Develop an inexpensive dynamic, real time analytical capability for multicomponent vapor quantification

DESCRIPTION: Chemical defense research involves the quantitative study of response – be it a sensor system or a host model – to a known concentration/dose of the threat agent in a vapor or aerosol form. It is increasingly important to be able to understand the dose-response characteristics of the subject in real time. To date, instrumentation integrated with such experiments has been either operated in batch vs. real time mode so that only static exposure data is retrieved, or is complex and prohibitively expensive. New developments in optical analysis techniques may afford a less expensive alternative to gas chromatography/mass spectrometry methods for such applications while bringing down the acquisition and operating costs associated with the analysis. Specifically, new capabilities in infrared detection based on laser photoacoustic and/or cavity ringdown phenomena have demonstrated real time detection and analysis performance at the part per trillion analyte level.

PHASE I: The Phase I feasibility/proof-of-concept study will thoroughly analyze and model the component and system level performance of an optical analyzer based on laser photoacoustic and/or cavity ringdown spectroscopy in order to develop an end-to-end theoretical performance model and to drive the preliminary design constraints for a Phase II demonstration system.

PHASE II: The Phase II effort will fabricate, integrate, test, and optimize the performance of a real-time gas analysis prototype platform based on the outcome of the Phase I feasibility study.

PHASE III Dual Use Applications: A real time, multicomponent analytical capability is needed for the advancement of knowledge and insight into chemical warfare agent exposure in real time while affording simultaneous knowledge and insight into interactions between target analytes and environmental species. The capability to quantitatively define the concentration of gas phase constituents in real time is critical to new product development in the pharmaceutical, semiconductor, and advanced materials industries.

REFERENCES:
1. Scotoni, M.; Rossi, A.; Bassi, D.; Buffa, R.; Iannotta, S.; Boschetti, A., "Simultaneous detection of ammonia, methane and ethylene at 1.63 mm with diode laser photoacoustic spectroscopy", Applied Physics B: Lasers and Optics, 82(3), 495-500, 2006.

2. Pushkarsky, Michael; Tsekoun, Alexei; Unayevskiy, Ilya G.; Go, Rowel; Patel, C. Kumar N. "Sub-parts-per-billion level detection of NO2 using room-temperature quantum cascade lasers", Proceedings of the National Academy of Sciences of the United States of America, 103(29), 10846-10849, 2006.

3. Tittel, Frank K.; Bakhirkin, Yury; Kosterev, Anatoliy A.; Wysocki, Gerard, "Recent advances in trace gas detection using quantum and interband cascade lasers", Reza Kenkyu, 34(4), 275-282, 2006.

4. Grossel, Agnes; Zeninari, Virginie; Joly, Lilian; Parvitte, Bertrand; Courtois, Daniel; Durry, Georges, "New improvements in methane detection using a Helmholtz resonant photoacoustic laser sensor: A comparison between near-IR diode lasers and mid-IR quantum cascade lasers", Spectrochimica Acta, Part A: Molecular and Biomolecular Spectroscopy, 63A(5), 1021-1028, 2006.

5. Elia, A.; Rizzi, F.; Di Franco, C.; Lugara, P. M.; Scamarcio, G., "Quantum cascade laser-based photoacoustic spectroscopy of volatile chemicals: Application to hexamethyldisilazane", Spectrochimica Acta, Part A: Molecular and Biomolecular Spectroscopy, 64A(2), 426-429, 2006.

6. Bakhirkin, Y.A.; Kosterev, A.A.; Curl, R.; Tittel, F.K.; Yarekha, D.A.; Hvozdara, L.; Giovannini, M.; Faist, J. Sub-ppbv nitric oxide concentration measurements using cw thermoelectrically cooled quantum cascade laser-based integrated cavity output spectroscopy. Appl. Phys. B, 82, 149-154, 2006.

KEYWORDS: vapor detection, vapor quantification, optical spectroscopy, photoacoustic, laser, cavity ringdown, trace analysis

CBD08-105 TITLE: Multiple Indication Adjuvants

TECHNOLOGY AREAS: Chemical/Bio Defense, Biomedical

OBJECTIVE: Develop immunological adjuvants that demonstrate efficacy in conjunction with various types of vaccine formulations designed against Category A and B potential biowarfare agents. Ideally, these adjuvants would be efficacious in augmenting both humoral and cellular immune responses in conjunction with vaccines designed for pre- and/or post-exposure prophylaxis.

DESCRIPTION: Vaccination strategies are a first line of defense against microbial pathogens utilized as bioweapons. However, the identification of safe and effective vaccines to combat lethal biowarfare agents has proven to be an elusive and costly endeavor. Often, substantial resources are dedicated to testing a large number of vaccines only to identify vaccines that meet threshold performance criteria in small animals, yet are ineffective at stimulating a protective immune response in humans. Additionally, the current stockpiles of vaccines to biowarfare agents are underrepresented and the number of doses is severely insufficient. Adjuvants are routinely used to augment the immune response to vaccines. In humans however, most approved adjuvants produce only a modestly enhanced immune response and are often associated with negative side effects. Developing a safe, versatile adjuvant that enhances both humoral and cellular immunity, as well as augments immune responses in conjunction with multiple types of vaccines would greatly enhance the ability to protect the warfighter from weaponized biological pathogens.

PHASE I: Design an adjuvant to be co-administered with a characterized vaccine against a pathogen of interest to the Dodd Joint Chemical and Biological Defense Program. Show proof of concept as well as feasibility by demonstrating that both the humoral and cellular immune responses are augmented in small animals following a primary immunization with the adjuvant plus chosen vaccine. Criteria should not only include the magnitude of the response (ex: Ab titer, T cell activation, cytokine production), but also time of onset. Demonstrate that the effectiveness of the adjuvant is not vaccine specific by employing a panel of vaccines against different pathogens, as well as vaccines of distinct formulations (i.e., whole organism, protein, DNA, etc.).

PHASE II: Demonstrate the efficacy of the adjuvant in challenge studies conducted in small animal models. Show that the augmented humoral and cellular immune response generated following a primary immunization of small animals with the designed adjuvant plus chosen vaccines (phase I) translates to improved protection against challenge by the appropriate pathogens. Examine efficacy of the adjuvant in both pre- and post-exposure prophylaxis when appropriate. Perform pharmacology and toxicology studies of the adjuvant in animal models. This data may be used to support an IND application.

PHASE III DUAL USE COMMERCIALIZATION: Successful Phase II products can be used in conjunction with vaccines for the warfighter against bioweapon threats. In addition, these adjuvants can be used as a medical countermeasure against any pathogen that may strike the general population. Phase III and IND approval would lead to appropriate clinical trials to gain FDA approval that may be funded through additional government and/or private funding sources.

REFERENCES:
1. Barr,TA, Carling J, and Heath AW. (2006) Co-Stimulatory agonists as immunological adjuvants. Vaccine 24 (17): 3399-3407.

2. Rosenthal, KS and Zimmerman, DH. (2006). Vaccines: all things considered. Clin. Vaccine Immunol. 13(8): 821-9.

3. Hodge, JW et al., (2006). Costimulatory molecules as adjuvants for immunotherapy. Front. Biosci. 11:788-803.

KEYWORDS: Adjuvants, Vaccines, Biowarfare, Humoral immunity, Cellular Immunity

CBD08-106 TITLE: <u>Development of Biomolecules for Countering Blockage of Neurotransmitter Release by Botulinum Poisoning</u>

TECHNOLOGY AREAS: Chemical/Bio Defense, Biomedical

OBJECTIVE: Identify small organic and bio- molecules which can enhance neurotransmitter release in botulinum poisoned nerve cells. This will be accomplished by developing a technology to target various drug candidates to these nerve cells.

DESCRIPTION: Long-lasting paralysis is caused by the persistence of endopeptidase activity of botulinum neurotoxin (BoNT) within poisoned cells. The focus of botulism therapy is to rescue poisoned cells by blocking this endopeptidase activity, which is critical for restoration of muscular function. Recent therapies under development for botulism include antibody-based and small molecule inhibitors. The antibody-based therapy largely targets circulating BoNTs; therefore the window to administer such a drug is short. A passive immunization for prophylaxis may be possible, but further development is necessary. Therefore, new methods are needed to counter the effects of BoNT endopeptidase activity inside the poisoned cells. For instance, it may be possible to augment the host cell response to counter the toxic effect, by methods such as increasing the cellular proteins which are targets of the endopeptidase.
In addition, a major obstacle in botulism therapy is the ability to deliver the endopeptidase inhibitors to the cytoplasm of these nerve cells. Hence, technologies that can specifically target and transverse BoNT poisoned cells will be useful. Unique and novel drug candidates in addition to targeting specific botulinum affected cells will need to be identified and developed to counter the effects of BoNT endopeptidase.

PHASE I: Develop therapeutic delivery molecules by identifying affected cell targeting elements. Identify bioactive molecules for countering BoNT endopeptidase activity within the cells. Demonstrate delivery of small and large molecules into neuronal cells.

PHASE II: Demonstrate delivery of bioactive molecules into botulinum poisoned nerve cells using labeled molecules. Demonstrate functional efficacy of delivering drug molecules against BoNT to these nerve cells, by decreased toxicity and enhanced neurotransmitter release.

PHASE III DUAL USE COMMERCIALIZATION: This phase will use appropriate animal models to establish efficacy of the developed therapeutic(s). In addition, if successful, will begin to establish initial drug formulation, stability, specificity, and other IND enabling characteristics of target drug candidates using cell culture tissues and nerve-muscle junction preparations. Therapeutics against botulinum will be useful to the Dodd in the case of botulinum used as a bioweapons agent. In addition, botulism poisoning, although rare, is also a concern for the general population, due the natural presence of the bacterium which causes the disease.

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KEYWORDS: Botulinum, botulism, drug delivery, therapeutic, nerve cell, toxin

CBD08-107 TITLE: <u>Development of New General Modalities of Effective Drug Delivery to the Central Nervous System</u>

TECHNOLOGY AREAS: Chemical/Bio Defense, Biomedical

OBJECTIVE: Objective of this topic is to develop new modalities of effective drug delivery to Central Nervous System (CNS) for the treatment of toxic chemical induced or combat related brain injuries. Emphasis is placed on innovative technologies that can deliver a different approved therapeutic drug to the CNS or being developed therapeutic drugs to the CNS with minimal side effects. That is, a generic carrier for brain delivery of a wide class of drugs.

DESCRIPTION: Drug delivery to the brain is limited by the blood-brain barrier (BBB), which markedly impacts treatment for a vast number of central nervous system related injuries in military medicine. This has impeded CNS drug development and treatment. Therefore, discovery of new modalities of effective drug delivery to the CNS is of great importance for treatment of brain injuries, spanning the realm of brain injuries resulting from cholinergic overload as a result of organophosphate toxicity to treatments for traumatic head wound.
The factors that determine penetration of substances from the blood to the CNS are lipid solubility, molecular size, and charge. Since few drugs cross the BBB, the development of BBB drug delivery technologies is a high priority for the treatment of brain injuries. This program will develop new and innovative targeting technology by enhancing the delivery of neuroprotectants such as oximes, cyclosporine A, adenosine receptor agonists, or nerve agent bioscavenger for effective protection against chemical warfare agents or combat related brain injuries.

PHASE I: Evaluate approximately 3-6 technologies of choice (example, nanoparticle, liposome, or compounds that enhance BBB permeability) for enhanced delivery of brain pretreatments or therapeutics in an appropriate animal model.

PHASE II: Demonstrate the efficacy of the most efficacious technology with multiple therapeutic agents in laboratory animals exposed to nerve agents. Animal exposure to toxic agents could be performed at several labs at institutions having the appropriate biosafety level. Extend the studies to higher order animals and prepare for clinical trials.

PHASE III / DUAL APPLICATION: The new lead candidate will be initiated into clinical trials, possibly in collaboration with pharmaceutical or biotechnology companies for Food and Drug Administration (FDA) review of safety and efficacy. Because of the high commercial potential of successful methods, this phase also involves a commercialization strategy to market the technology to major pharmaceutical firms.

Efficient BBB delivery agents against organophosphate induced and combat related brain injuries will have important application not only meeting Dodd objectives for chemical/biological terrorism and combat, but also civilians accidentally exposed to pesticides or involved in terrorist events, or suffering from emergency head injuries. More than 23,000 emergency room visits per year in the United States can be accounted for pesticide poisoning and 100,000 or more visits to emergency rooms result from head injuries from car accidents and falls.

KEYWORDS: Blood brain barrier, neuroprotectant, brain injury, drug delivery, central nervous system

CBD08-108 TITLE: <u>Scavenging of Organophosphates by Synthetic Oligonucleotides</u>

TECHNOLOGY AREAS: Chemical/Bio Defense, Biomedical, Human Systems

OBJECTIVE: Develop stable catalytically active oligonucleotides (CAO), either DNAzymes or Ribozymes, capable of converting post-exposure, organophosphates such as soman to a non-toxic form in vivo. These CAO need to be stable in vivo and have a high turnover rate.

DESCRIPTION: In 1982, the world of catalysis was broadened with the discovery of a RNA molecule that was self-splicing. Since the initial discovery of these catalytic RNA molecules called ribozymes, ribozymes have been reported with additional catalytic activities. Naturally occurring ribozymes have been found that catalyze phosphoester hydroysis. Synthetic DNA molecules with a similar catalytic activity have also been isolated starting with a “population of random-sequence DNA molecules”. Since DNAzymes are capable of cleaving phosphoesters in RNA and DNA, a CAO might be found to cleave the phosphoester in G agents, and soman in particular. This CAO must have a reasonable turnover rate (100 organophosphate molecules per CAO) and a reasonable catalytic rate (1 molecule/sec). With these threshold values, 300 nanomoles of CAO can hydrolyze 600 micrograms of soman in two minutes. Further, the CAO may require modification to produce an in vivo half life, post-exposure, of one hour.

PHASE I: Develop a method to select CAO capable of degrading organophosphates in vitro, such as soman in particular. Select a population of catalytic molecules and determine their turnover rate and catalytic rate under laboratory conditions. Verify that the products are non-toxic.

PHASE II: Optimize the catalytic activity by iterative methods. Modify the CAO to have an in vivo half life of one hour. Test if the modified CAO can spare cells in tissue culture from that activity of G agents and soman in particular. Testing may require access to a chemical surety facility.

PHASE III DUAL USE APPLICATIONS: If successful, this innovative approach has significant applicability to DoD, Dept. of Homeland Security, and emergency medicine. Further, these products have the potential to treat accidental organophosphate poisoning.

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KEYWORDS: Ribozyme, DNAzyme, organophosphates, G-Agents, non-bioscavengers, catalytically active oligonucleotides, soman